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Two topics will be discussed: (A) the phenomena of cell dependence upon narcotics and tolerance to these compounds, and (B) the problem of how neurons form synaptic connections with specificity. ~~The same~~ mouse neuroblastoma x rat glioma hybrid cell line ~~was used~~, was used as a model system for studies on both topics. This cell line is one of many somatic cell hybrid and neuroblastoma cell lines which were established during the past decade and characterized with respect to neural properties in the search for experimentally advantageous model systems for problems in neurobiology. The results which gradually emerged from these studies show that genes for enzymes of neurotransmitter synthesis, receptors, and action potential ionophores which are characteristically expressed by non-dividing neurons, also can be expressed by clonal lines of cells, that the parental programs of gene expression are inherited and thus can be perpetuated in a fairly stable fashion, and that somatic cell hybrids can be generated which either have acquired new neural properties not expressed by the parental cell lines or which have lost neural properties. The new programs of gene expression which have been generated also are inherited and thus can be perpetuated easily.

Fusion of clonal neuroblastoma cells with normal embryonic neuroblasts or neurons ~~would appear to be~~ ^a particularly promising approach for, in effect, genes expressed by developing neurons can be rescued and may continue to function in clonal hybrid cell lines. For example, fusion of clonal mouse neuroblastoma cells with mouse embryo sympathetic ganglion neurons yielded a hybrid cell line which had acquired a set of neural properties which were not found with the neuroblastoma parental cells, such as tyrosine hydroxylase activity, small dense core vesicles, and excitatory muscarinic acetylcholine receptors. Similarly, fusion of clonal mouse neuroblastoma cells with clonal rat glioma cells yielded hybrid cell lines such as NG108-15, which have choline acetyltransferase activity, clear vesicles 500 A in diameter and excitatory muscarinic acetylcholine-receptors; properties not found with the parental cell lines.

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Most neural properties of clonal cells which have been examined thus far seem to be regulated by environmental factors. Populations of cells can be shifted in synchrony from a poorly differentiated, neuroblast-like state to a well-differentiated, neuron-like state by either selecting for non-dividing cells, decreasing the rate of cell division, or increasing intracellular levels of cAMP.

Neuroblastoma x glioma NG108-15 hybrid cells also synthesize stereospecific high affinity morphine receptors. The average cell has 3×10^5 opiate receptors; whereas, the average parental neuroblastoma cell has approximately 0.6×10^5 opiate receptors. Opiate receptors were not detected with glioma parental cells.

Morphine and other opiates reduce cAMP levels of intact neuroblastoma x glioma hybrid cells and inhibit basal and PGE_1 -stimulated adenylate cyclase activity in homogenates. The effects of opiates are reversed completely by the narcotic antagonist, naloxone, which competes with narcotics for sites on the opiate receptor but lacks the pharmacologic activities of opiates. The relative affinities of narcotics for the opiate receptor agree well with their effectiveness as inhibitors of adenylate cyclase and with their pharmacologic potency. Morphine sensitive and insensitive cell lines were found and the degree of sensitivity to morphine was shown to be related to the abundance of opiate receptors.

A new class of peptides, the endorphins or enkephalins, discovered in 1975 by Hughes and Kosterlitz and associates, possess properties of morphine and other opiates and are present in mammalian pituitary and in certain neurons. A pituitary prohormone, β -lipotropin, is thought to be a precursor of both the endorphins and β -melanotropin. Endorphins are derived from the carboxy-terminal portion of β -lipotropin (residues 61-91) and β -melanotropin from residues 41-58. The smallest fragment of β -lipotropin which retains opiate activity is methionine-enkephalin which corresponds to β -lipotropin (61-65).

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Met-enkephalin is the most potent peptide inhibitor of adenylate cyclase known, for the concentration required for half-maximal inhibition of adenylate cyclase is 11 nM.

We wondered whether exposure of cells to an opiate for hours or days would result in cell dependence or tolerance to the opiate. The results showed that growth of NG108-15 cells in the presence of morphine or met-enkephalin for 12 to 48 hours results in an inhibition of adenylate cyclase and in a slow, compensatory increase in adenylate cyclase activity which is delayed in onset but then can be expressed in the absence of the opiate and is relatively long-lived. Cells then have normal cAMP levels and appear tolerant to the opiate because the increase in adenylate cyclase activity is approximately equal to the inhibition of enzyme activity by the opiate. However, the cells are dependent upon the opiate to maintain normal cAMP levels. If morphine is withdrawn or displaced from the opiate receptor by the opiate antagonist, naloxone, the enzyme is no longer inhibited and abnormally high adenylate cyclase activity is revealed. Dual regulation of adenylate cyclase by opiates thus accounts for the phenomena of narcotic dependence and tolerance. Thus opiate peptides and narcotics can act as pleiotropic regulators of the cells response to neurotransmitters and hormones which are coupled to the activation of adenylate cyclase. Opiates thus alter the preception of neurons to incoming messages which are destined for adenylate cyclase.

NG108-15 hybrid cells also possess α -adrenergic receptors and excitatory muscarinic acetylcholine receptors which also are coupled to the inhibition of adenylate cyclase. Cells were cultured in the presence of norepinephrine or carbamylcholine for 10-48 hours, then the effects of withdrawal of the receptor ligand either by replacing the medium or by the addition of a receptor antagonist

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was tested. Withdrawal of norepinephrine or carbamylcholine resulted in 9- and 5-fold increases in cAMP levels of intact cells respectively, Adenylate cyclase activity also increases but to a lesser extent. Studies on the specificity of receptor antagonists showed that the inhibitions of adenylate cyclase by norepinephrine, acetylcholine or opiates are mediated by different species of receptors. These results show that dual regulation of adenylate cyclase is a general phenomenon and that cells become dependent upon norepinephrine and acetylcholine as well as opiates. The cells develop an apparent tolerance but in fact remain sensitive to the compound. NG108-15 hybrid cells synthesize, store, and excrete acetylcholine and generate action potentials in response to electrical stimulation. When the hybrid cells were cocultured with striated muscle cells which have abundant nicotinic acetylcholine receptors, they were found to form synapses with muscle cells in abundance.

Studies on the embryonic development of the mammalian neuromuscular synapse reveal that at an early developmental stage when nicotinic acetylcholine receptors are distributed over the entire surface of the muscle cell, a single muscle cell usually is innervated by multiple neurons. At this stage transmission across the synapse is relatively inefficient since some, but not all, neuron action potentials evoke muscle responses. As the synapse matures the number of synaptic vesicles which release acetylcholine per motor neuron action potential increases 100-300 fold with a concomitant increase in the efficiency of transynaptic communication, and the concentration of nicotinic acetylcholine receptors decreases at all sites on the muscle cell other than the site of one synapse. Thus, the muscle cell is converted from a permissive state with respect to synapse formation to a nonpermissive state, and each muscle cell then is innervated by only one motor neuron.

The synaptic connections which form between NG108-15 hybrid cells and cultured striated muscle cells closely resemble the synapses which form between

normal motor neurons and muscle cells at an early developmental stage. Although some NG108-15 action potentials evoke muscle action potentials, most muscle responses are between the threshold for initiation of action potentials.

Little is know at the molecular level about the process of synapse formation and the basis for the specificity of synaptic connections between cells. Two kinds of hypothesis have been suggested to account for synapse specificity: (1) that cells prior to synapse formation complimentary molecules are present on the surface of neurons and their synaptic partners which must interact correctly prior to synapse formation; i.e., a molecular cell recognition code for synapse formation, or (3) synaptic circuits assemble with little specificity and then those which function appropriately are maintained by a process of selection whereas the others disappear.

This clonal hybrid cell line provides an opportunity to test the specificity of synapse formation. If the formation of synaptic connections between neurons and muscle cells were coded by specific cell recognition molecules, then discrete classes of muscle cells with different specificities for synpase formation might be expected. However the results revealed that NG108-15 cells form synapses with clonal muscle cells and with myotubes from different embryonic muscles, and from different organisms such as the chick, mouse and rat. Most of the muscle cells tested were innervated by hybrid cells. The results strongly suggest that most striated muscle cells have the same specificity for synpase formation. No evidence was found for discrete classes of muscle cells with different specificities for synapse formation. In essence the results suggest that the formation of the neuromuscular synapse is not dependent upon a cell recognition code. The results also indicate that mechanisms of synapse formation are conserved during evolution and thus may be largely universal.

The evidence suggest that much of the specificity of the normal neuro-

6

muscular synapse is acquired after synapses form by a process of selection which reduces the number of synapses and which is dependent upon effective transmission across the synapse, rather than by a process of matching complimentary molecules on neurons and muscle cells which code for different synaptic connections. It seems likely that the amount of acetylcholine excretion from the neuron and the distribution of nicotinic acetylcholine receptors on the muscle cells are regulated and that both are involved in the synapse selection process.